PATENT
Our Docket: 066654.669 (P-LJ 4859)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ruoslahti and Pasqualini	)	Group Art Unit: 1632
Serial No.: 09/922,227	)	Examiner: Scott D. Priebe
Filed: August 2, 2001	)	
For: METHODS OF IDENTIFYING MOLECULES THAT HOME TO A SELECTED ORGAN *IN VIVO*	)	

Commissioner for Patents
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Erkki Ruoslahti, declare as follows:

1) I am the Erkki Ruoslahti who is named as a co-inventor of the above-identified patent application.

2) I understand that the claims of the subject application stand rejected, in part, as one skilled in the art allegedly would not have been able to identify homing molecules by *in vivo* panning with untagged libraries.

3) I believe that in 1995, at the time the priority application for the above-identified application was filed, an ordinary scientist would have been able to use untagged peptide or small molecule libraries in the claimed *in vivo* panning methods to recover and identify molecules that selectively home to a selected organ or tissue.

4) Corroboration of the ability to identify homing molecules using an untagged small molecule library is provided herein in Exhibits 1 to 6, which demonstrate identification of homing molecules in murine brain and lung extracts following intravenous injection of a small molecule library.

5) In particular, a diverse library of about 200 compounds was prepared by routine chemical synthesis of the individual compounds derived mainly from scaffolds commonly found in known drugs. A second library prepared by randomly selecting ten different compounds from the first library was dissolved in Dulbecco's phosphate buffered saline (PBS), with each molecule at a final concentration of 2.5 mM. These compounds were analyzed individually by mass spectrometry to obtain an experimental mass for each molecule. The structures and calculated molecular weights of the members of the library are shown in Table 1.

6) To identify molecules that home to a selected organ, an anesthetized 2-month old female Balb/c mouse was injected intravenously in the tail vein with 100 µl of the library. After 5 minutes, the lungs, liver, kidney, and brain were isolated, washed with 5 ml of PBS to remove excess blood, weighed, and trimmed into small pieces with scissors. Each organ was mixed with 10 ml of acetone and treated by at least 30 strokes of a Dounce homogenizer. The organ/acetone mixtures were transferred to 15 ml Corning centrifuge tubes and incubated at -20°C for 12 hours to precipitate proteins. Following centrifugation at 2,500x*g* for 20 minutes at 4°C, supernatants were recovered and dried in a vacuum for 12 hours. A similar set

of organ extracts, prepared from mice injected with 100 µl of PBS, served as an internal control for the experiment.

7) Organ extracts were analyzed by mass spectrometry. Dried organ extracts were dissolved in 50 µl of acetonitrile and diluted 1:2 in 20 mg/ml alpha-cyano-4-hydroxycinnamic acid (HCCA) in 50% acetonitrile/0.1% trifluoroacetic acid before analysis of a portion of the diluted extract on an Applied Biosystems MALDI-TOF Voyager DE-Pro mass spectrometer. Masses of individual compounds and compounds in organ extracts of PBS-injected mice were compared to compounds in organ extracts from the library-injected mice to identify molecules in the library that selectively homed to a particular organ.

8) The mass spectrometric results demonstrate that molecules 1B5 and 2C11 accumulated in brain upon intravenous injection (Figure 1) but did not home to lung or liver (Figures 2 and 3). Similarly, the mass spectrometric results demonstrate that molecules 2E3 and 1B12 accumulated in lung and that molecule 1B12 accumulated in both lung and liver (Figures 4 and 5), but that these molecules did not home to brain (Figure 6).

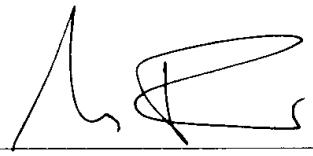
9) These results agree with results in the literature showing that molecule 2C11, identified in the above experiments as a molecule that selectively homes to brain, is a pharmacologically active benzodiazepine (Oxazepam; Serax) known to cross the blood-brain barrier. In contrast, molecule 2A6, a pharmacologically inactive benzodiazepine, was not observed to home to brain in our experiments.

10) In sum, these results corroborate that only routine techniques would have been required to inject a mixture of untagged small molecules into an animal and to recover and identify the small molecules that selectively accumulate in a selected organ or tissue.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

4-22-03

Date



Erkki Ruoslahti

TABLE 1

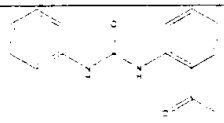
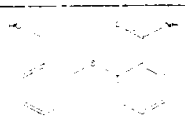
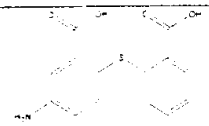
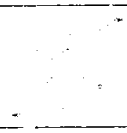
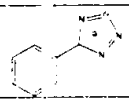
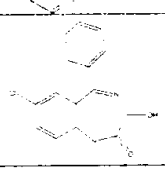
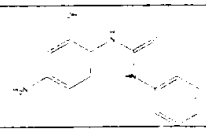
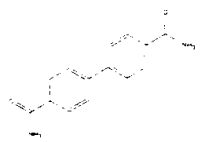
COMPOUND	STRUCTURE	MOLECULAR WEIGHT
1E4		226.2774
1B5		273.349
1B6		289.3052
1D6		240.3012
1B12		240.2148
2A6		264.3262
2C11		300.7439
2D8		243.2646
1B1		240.261
2E3		225.2464

FIGURE 1

Compounds 1B5 and 2C11 localize to brain

PBS-injected

Library-injected

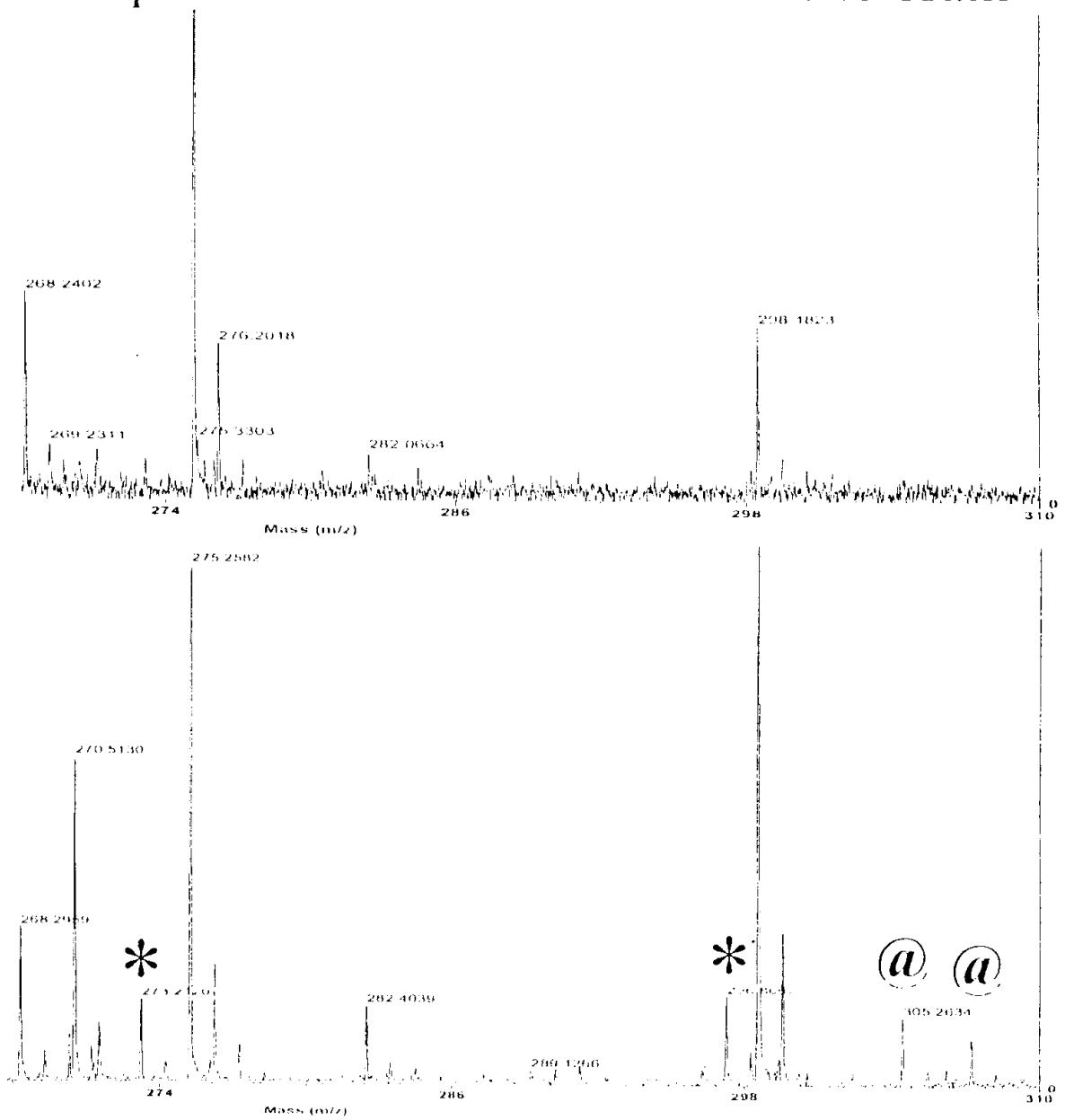


FIGURE 2

Compounds 1B5 and 2C11 are not detected in lung

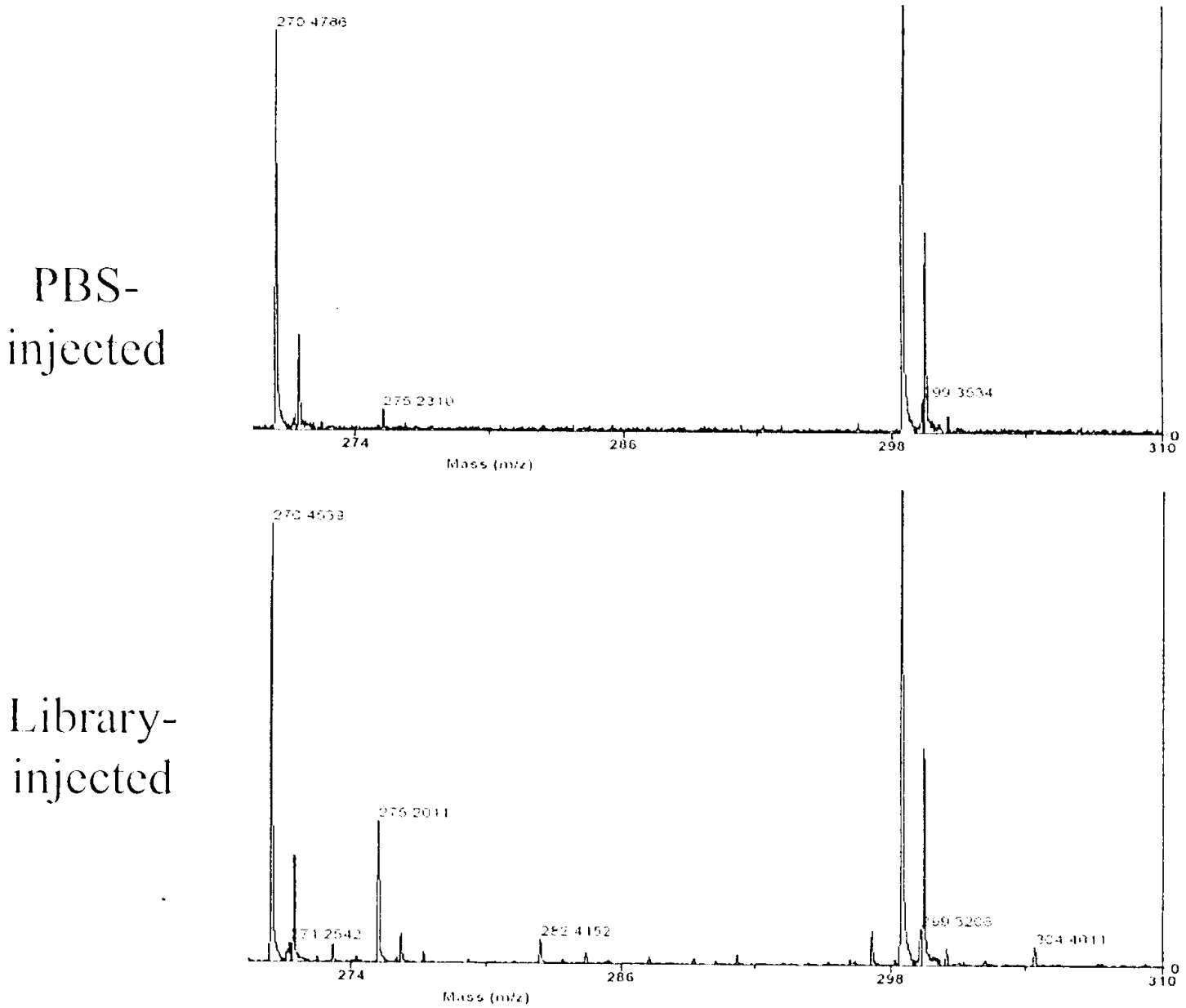
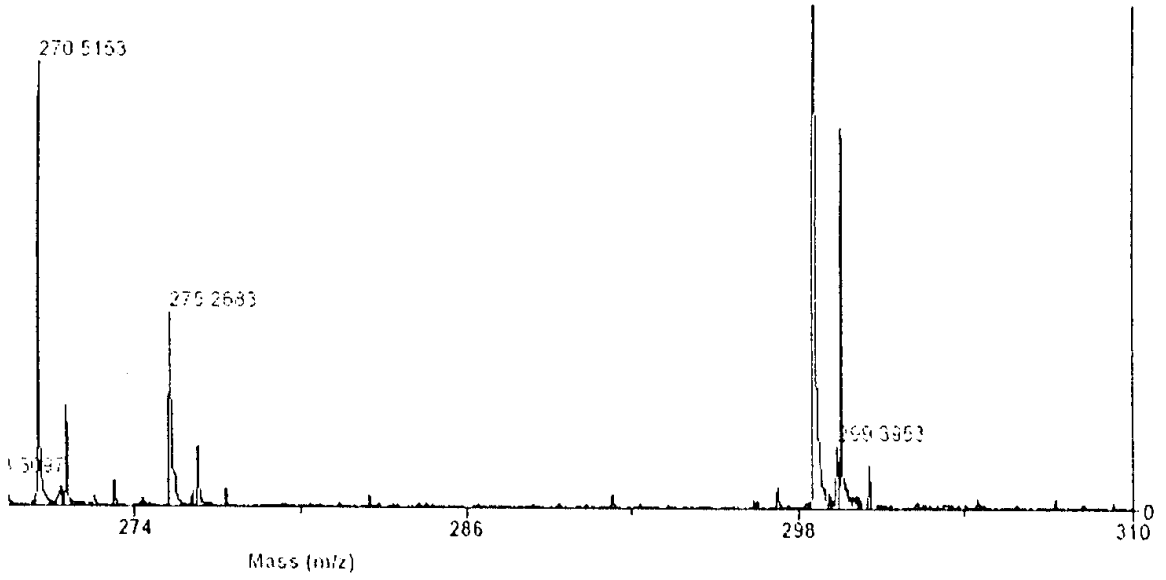


FIGURE 3

Compounds 1B5 and 2C11 are not detected in liver

PBS-injected



Library-injected

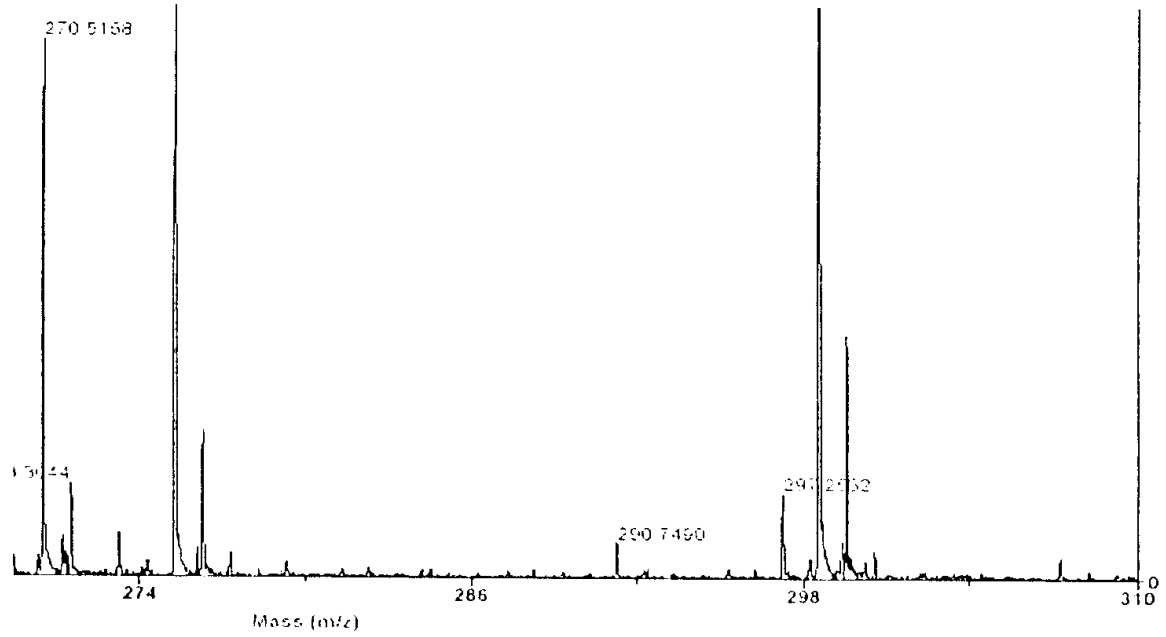


FIGURE 4

Compounds 2E3 and 1B12 localize to lung

* 2E3
@ 1B12

PBS-injected

Library-injected

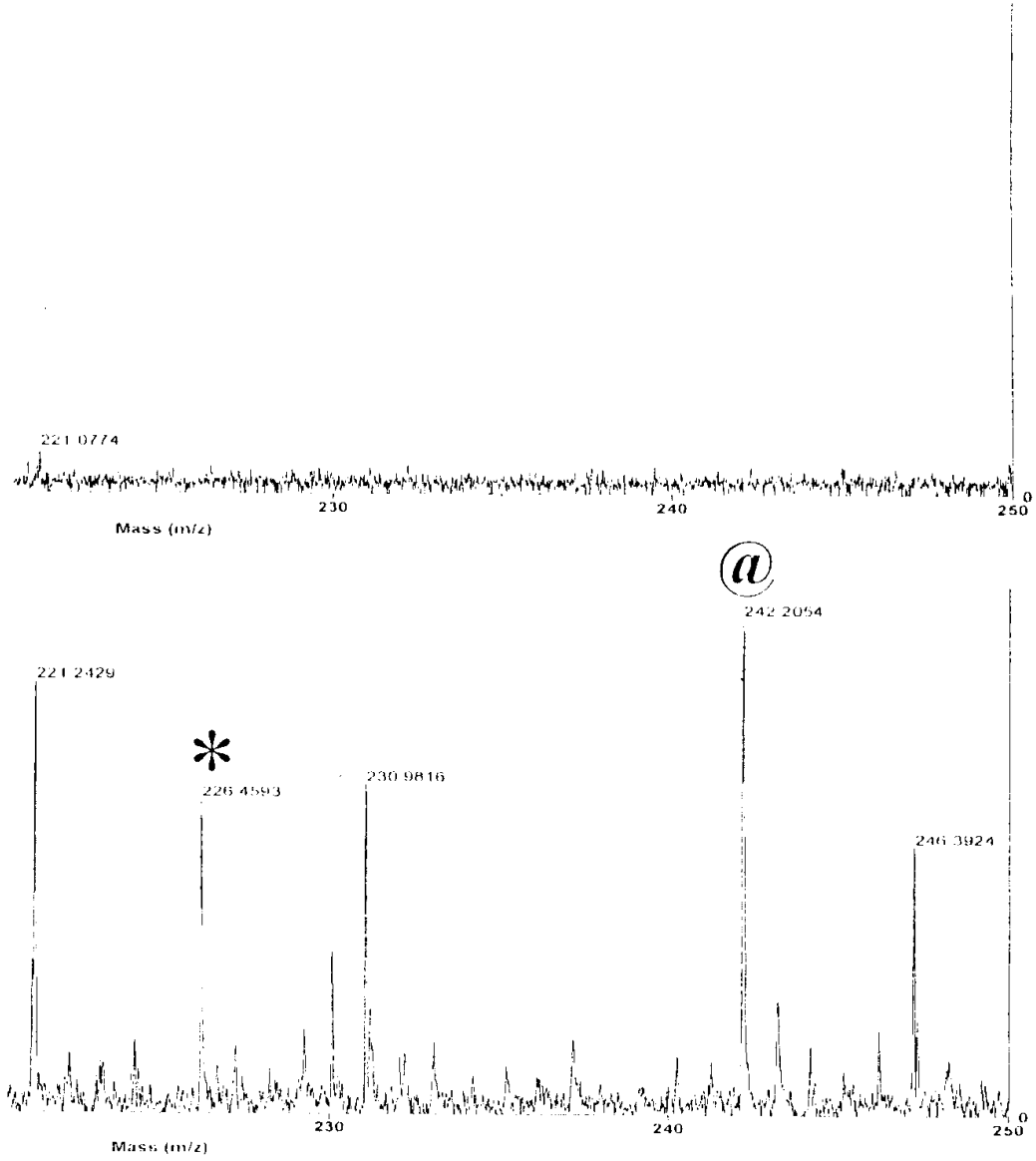
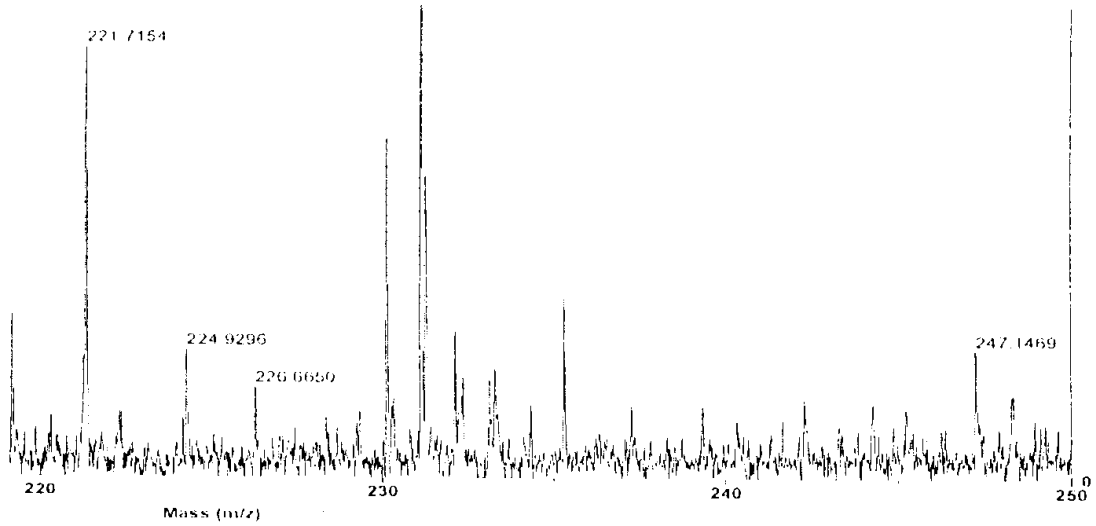


FIGURE 5

Compound 1B12 localizes to liver

PBS-injected



@ 1B12

Library-injected

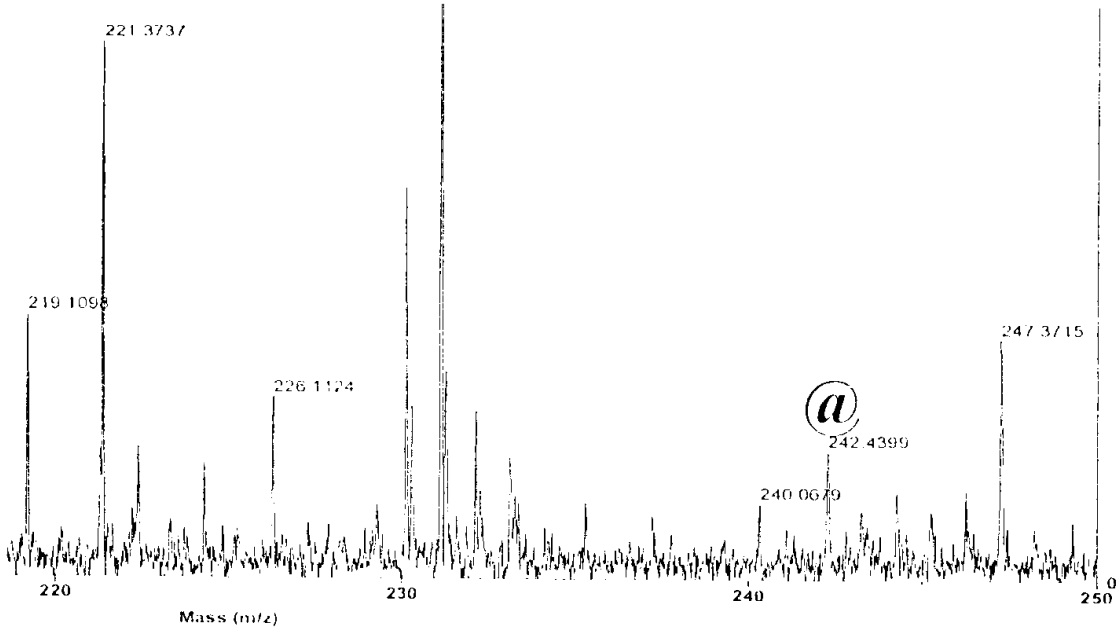
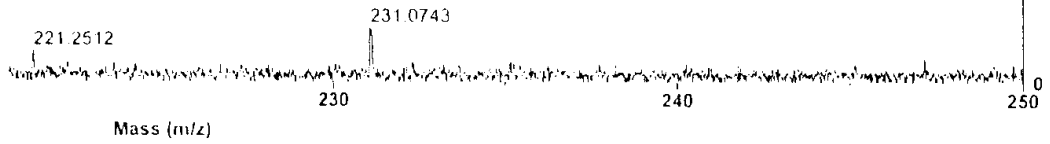


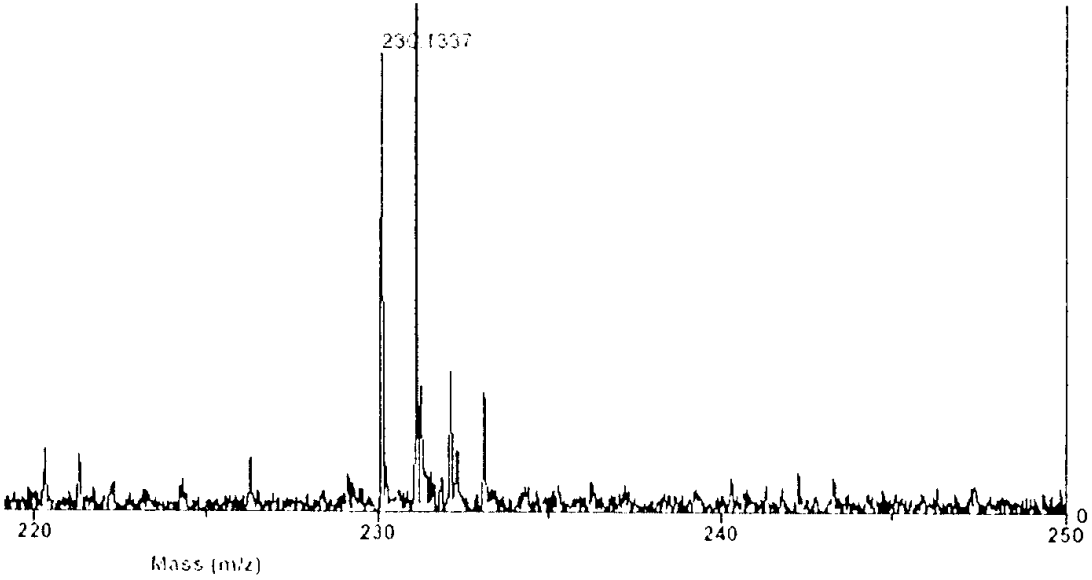
FIGURE 6

Compounds 2E3 and 1B12 are not detected in brain

PBS-injected



Library-injected



ONE EXECUTED DECLARATION PURSUANT TO 37 C.F.R. § 1.132, WITH ATTACHED TABLE 1 AND FIGURES 1-6 (11 pages)
Attorney Docket No.: 066654-669 (P-LJ 4859)
Serial No.: 09/922,227

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on April 22, 2003.

By Andrea L. Gashler
Andrea L. Gashler, Reg. No. 41,029

April 22, 2003
Date of Signature